

Figure 1: Stand alone Genome Threader results for INSP093 partial polypeptide sequence (SEQ ID NO:6).

Genome Threader results – Energy Scores

Rank	PDB Code	Norm Aln Score	Raw Aln Score	#Dbs Aligned	%Query Seq Aligned	Average Energy	Solvation Energy	Normal Net Score	%Compliance for Other	Front Res.	End Res.	Span Res.	Res for Target	Res for Thread	Aligned Length	Global Score
1	1qg7B00 (Aln)	30.58	53	25.0	78.8	44.7	-10.39	-2.31	0.928	65	50	117	1	52	68	0
2	1qg7-100 (Aln)	30.51	53	25.05	77.6	44.7	-10.54	-2.10	0.927	65	50	117	2	53	68	0
3	1qg7C00 (Aln)	28.52	60	13.4	17.2	69.8	8.64	2.81	0.897	54	47	143	36	128	97	0
4	2sl1-100 (Aln)	21.04	57	10.9	14.5	55.5	-25.57	-0.09	0.893	59	97	150	78	192	59	0
5	2sl1B00 (Aln)	26.47	60	45.2	2.6	18.4	-0.22	-2.31	0.896	54	107	134	603	625	31	0

Number 1: 1qg7, Stroma cell-derived factor-1alpha

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Figure 2: Alignment between INSP093 partial polypeptide sequence (SEQ ID NO:6) and the top hit, 1qg7.

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1qg7BB00 -----
          PVSLSYRCPCR-----FF
          :: : [S]
INSP093 msaqhglvskfglgllllgdkyfqrheqskphqeeidnlhspdlptpghPVTLHSLCFSPRGTLLEGPM
          10|    20|    30|    40|    50|    60|    70|
          20|    30|        40|    50|    60|
1qg7BB00 ESHVARANVKHLKILNTPN-----CALQI--VARLKNNNRQVCIDPklkwiqeylekaln-----
          ... .: .: .: .: .: .: .: .: .: .: .: .: .: .: .
INSP093 SSGFHRFEVENLRPQTAPKAGKGQMGERMARMATAKEGRPRCLDPglsrtphpgphvflphsptpasw
          80|    90|    100|    110|    120|    130|    140|
          150|
```

1qg7BB00 -----

INSP093 hqwapggtgwm~~l~~

Highlighted residues identify the conservation of cysteines between 1qg7 and INSP093.

10/540180

WO 2004/056859

PCT/GB2003/005621

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Figure 3: Stand alone Genome Threader results for INSP094 partial polypeptide sequence (SEQ ID NO:10).

Genome Threader results – Energy Scores

Num	PDB Code	Normal		Raw		SVD		MOnes		Enterprise		Solvation		Neural		GC Confidence		Emin_Pas		To Pos		Emin_Pos		To Pos		Alignment	
		Align	Score	Align	Score	SVD	Score	MOnes	Score	Enterprise	Score	Solvation	Score	Neural	Score	GC Confidence	Emin_Pas	To Pos	Score	Emin_Pos	To Pos	Score	Length	Global			
1	1ggi7880 [align]	30.58	53	25.0	78.8	44.7	10.39	-2.31	0.928	65	50	117	1	52	68	0	0	0	0	0	0	0	0	0	0	0	
2	Red-100 [align]	90.51	53	-25.0	77.6	44.7	10.54	-2.10	0.927	65	50	117	2	44	68	0	0	0	0	0	0	0	0	0	0	0	
3	1mam00 [align]	28.52	60	19.4	17.2	63.8	8.64	2.81	0.897	54	47	143	36	128	97	0	0	0	0	0	0	0	0	0	0	0	
4	1hi-100 [align]	27.04	57	10.9	14.5	35.5	3.57	-0.08	0.893	53	37	150	76	132	55	0	0	0	0	0	0	0	0	0	0	0	
5	2d1k00 [align]	26.47	60	45.2	2.6	38.4	-0.22	-2.31	0.896	54	307	134	603	625	31	0	0	0	0	0	0	0	0	0	0	0	

Number 1: 1hum, *Homo sapiens* macrophage inflammatory protein

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Figure 4: Alignment between INSP094 partial polypeptide sequence (SEQ ID NO:10) and the top hit, 1hum.

1humAA00	-----	apmgsdppta ^C FS
INSP094	ntendfyeicgnqshhhdnarikkklvdglefsqtmafsatkinmlfsqnhtirsifhsgfywgkg ^C CHK	: .
	10 20 30 40 50 60 70	
	20 30 40 50 60	
1humAA00	YTAR-KLPRNFVVDDYYETSSLCSQPAVVFQTKRSKQV-CADPSESWVQEyyvydlein	
INSP094	MSVHLFIHISN--RYFMTTSMCQEMAKIL---GRQIKCYLPTQSPVREsggktif-	
	80 90 100 110 120	

Highlighted residues identify the conservation of cysteines between 1hum and INSP094.

Figure 5: Nucleotide sequence of INSP094 with translation

1 cctggAACAT aattgacATT caataAAATAA ctgttGAATT aatttGTTGA attaatGACT
 61 caagAGTATT cacCTTTCAT acatAGGAAA tagaaTATAG aataCCGAGA atGATTtTA
 n t e n d f
 121 tgAGATCTGT ggAAATCAGT cacATCATCA cgACAAATGCA agaATAAAGA agTTAGTAGA
 y e i c g n q s h h h d n a r i k k l v
 181 tggcCTTGAG tttcccaAA caATGGCATT ttCTGCTACC AAAATAAATA tgTTATTcAG
 d g l e f s q t m a f s a t k i n m l f
 ←—————→
 INSP094-CP1

241 tcagaACCAC tggACTATAA gaAGTATATT ccATTCTGGT ttttACTGGG ggAAAGGATG
 s q n h w t i r s i f h s g f y w g k g
 301 ttGCCACAAG atgtcAGTCC atttATTcat tcATATATCC aATAGATATT ttATGACCAC
 c c h k m s v h l f i h i s n r y f m t
 361 ttccATGTGC caggAGATGG ctaAGATCCT tggAAAGACAG atAAAATGCT acCTACCAAC
 t s m c q e m a k i l g r q i k c y l p
 421 tcaaAGTCCA gttAGGGAGT caggGGGTAA aacaATATTc tagCACAGTG tgTTGAGGCC
 t q s p v r e s g g k t i f
 ←—————→
 INSP094-CP2

481 atggCATGGT gagCACAGGA gcCTGAAGGA gtggATGGAA aaggCACCTC agaATCATGA
 541 cactAGGAAA tgATTCTCAG t

Position and sense of PCR primers →

Figure 6: Nucleotide sequence with translation of PCR product cloned using primers INSP094-CP1 and INSP094-CP2

1 atggcatttt ctgctaccaa aataaatatg ttattcagtc agaaccactg gactataaga
m a f s a t k i n m l f s q n h w t i r
INSP094-CP1 →

61 agtatattcc attctggttt ttactggggg aaaggatgtt gccacaagat gtcagtccat
s i f h s g f y w g k g c c h k m s v h

121 ttattcattc atatatccaa tagatatttt atgaccactt ccatgtgccca ggagatggct
l f i h i s n r y f m t t s m c q e m a

181 aagatccttg gaagacagat aaaatgctac ctaccaactc aaagtccagt tagggagtc
k i l g r q i k c y l p t q s p v r e s

241 gggggtaaaa caatattcta gcaca
g g k t i f ← INSP094-CP2

Position and sense of PCR primers → ← →

Figure 7: Map of pCR4-TOPO-INSPO94

Molecule: pCR4-TOPO-INSPO94, 4222 bps DNA Circular

Type	Start	End	Name	Description
REGION	205	221	M13R	M13 rev priming site
REGION	243	262	T3	T3 priming site
MARKER	316		C CP2	INSPO94-CP2
REGION	559	295	C Insert	Inserted INSPO94-F2R2 PCR product
GENE	559	299	C cds?	INSPO94-F2R2 cds?
MARKER	559		C CP1	INSPO94-CP1
REGION	593	612	T7	T7 priming site
REGION	620	635	M13F	M13 for priming site
GENE	1424	2218	Kan	Kanamycin resistance gene ORF
GENE	2422	3282	Amp	Ampicillin resistance gene ORF
REGION	3427	4100	pUC ori	pUC origin

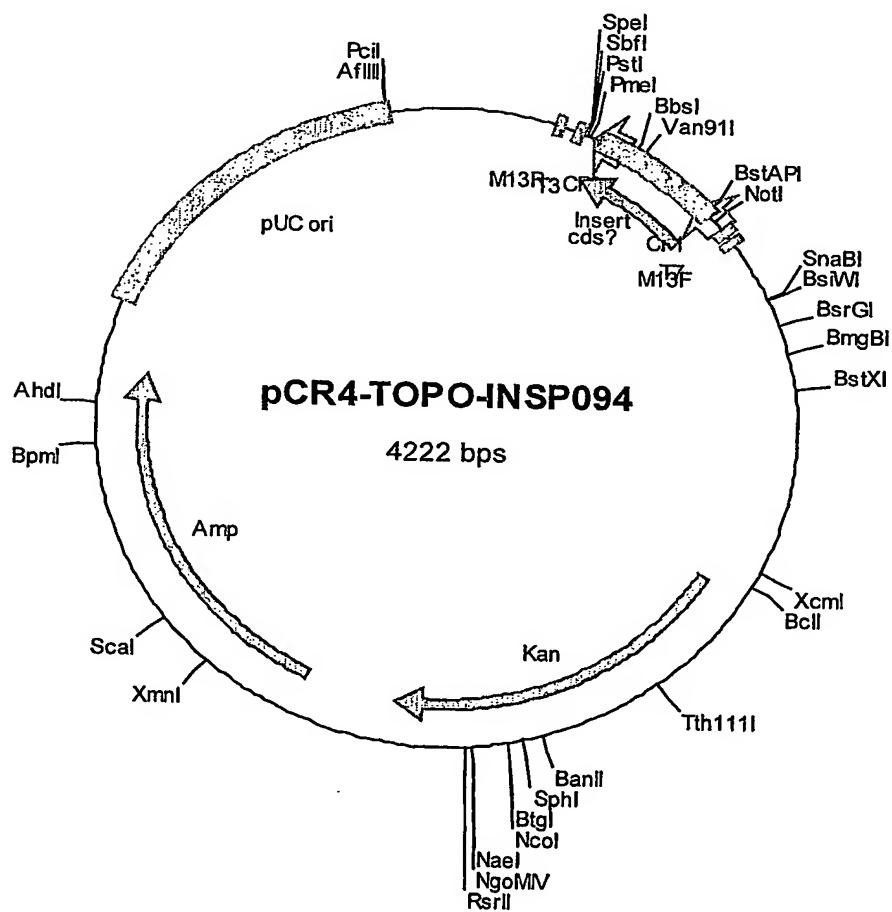


Figure 8: Map of pDONR 221

Molecule: pDONR221, 4759 bps DNA Circular
 File Name: pDONR221.cm5

Description:

Type	Start	End	Name	Description
REGION	295	268	C rrnB T2	
REGION	470	427	C rrnB T1	
REGION	536	553	21M13	
REGION	570	801	attP1	M13 Forward primer
GENE	1197	1502	ccdB	
GENE	1844	2503	Cm r	
REGION	2751	2982	attP2	
REGION	3040	3023	C M13 Rev	Chloramphenicol resistance gene
GENE	3153	3962	Kan r	
REGION	4083	4756	pUC ori	M13 Reverse primer

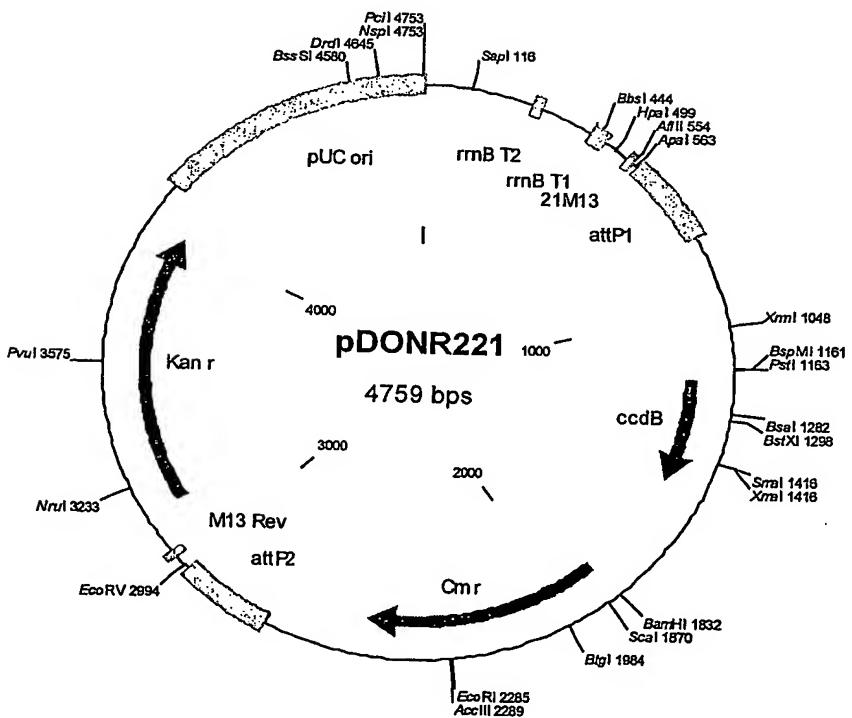


Figure 9: Map of expression vector pEAK12d

Molecule: pEAK12 d, 8760 bps DNA Circular
 File Name: pEAK12DEST.cm5

Description: Mammalian cell expression vector (plasmid ID 11345)

Molecule Features:

Type	Start	End	Name	Description
REGION	2	595		pmb-ori
GENE	596	1519	Amp	
REGION	1690	2795	EF-1alpha	
REGION	2703	2722		position of pEAK12F primer
REGION	2796	2845		MCS
MARKER	2855		attR1	
GENE	3256	3915	CmR	
GENE	4257	4562	ccdB	
MARKER	4603		C attR2	
REGION	4733	4733		MCS
REGION	4734	5162		poly A/splice
REGION	4819	4848	C	position of pEAK12R primer
GENE	5781	5163	C PUR	PUROMYCIN
REGION	6005	5782	C tK	tK promoter
REGION	6500	6006	C Ori P	
GENE	8552	6500	C EBNA-1	
REGION	8553	8752	sv40	

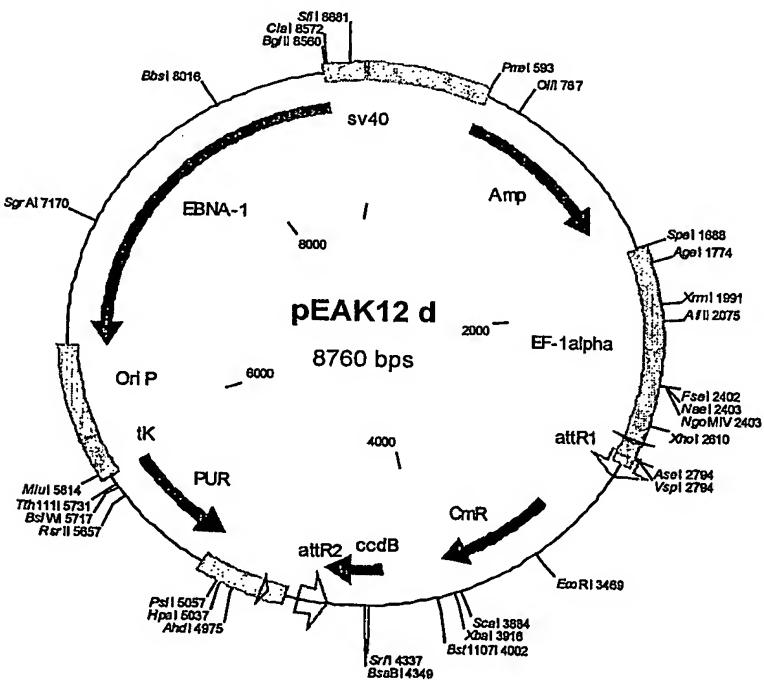


Figure 10: Map of expression vector pDEST12.2

Molecule: pDEST 12.2, 7278 bps DNA Circular
 File Name: pDEST12-2.cm5

Description: Eukaryotic expression vector

Type	Start	End	Name	Description
REGION	15	608	CMV	CMV promoter
MARKER	648		M13R	M13R primer
REGION	687	706	SP6	SP6 promoter
REGION	730	854	attR1	
GENE	963	1622	Cm	
GENE	1964	2269	ccdB	
REGION	2310	2434	attR2	
GENE	2484	2464	C T7	T7 promoter
MARKER	2512		C 21M13	21M13 primer
REGION	2784	3050	pA	SV40 polyadenylation signal
REGION	3176	3631	f1	f1 intergenic region
REGION	3791	4099	P SV40	SV40 ori & early promoter
GENE	4158	4952	Neo	
REGION	5016	5064	pA	synthetic poly adenylate signal
GENE	5475	6335	Amp	
REGION	6480	7153	ori	pUC ori

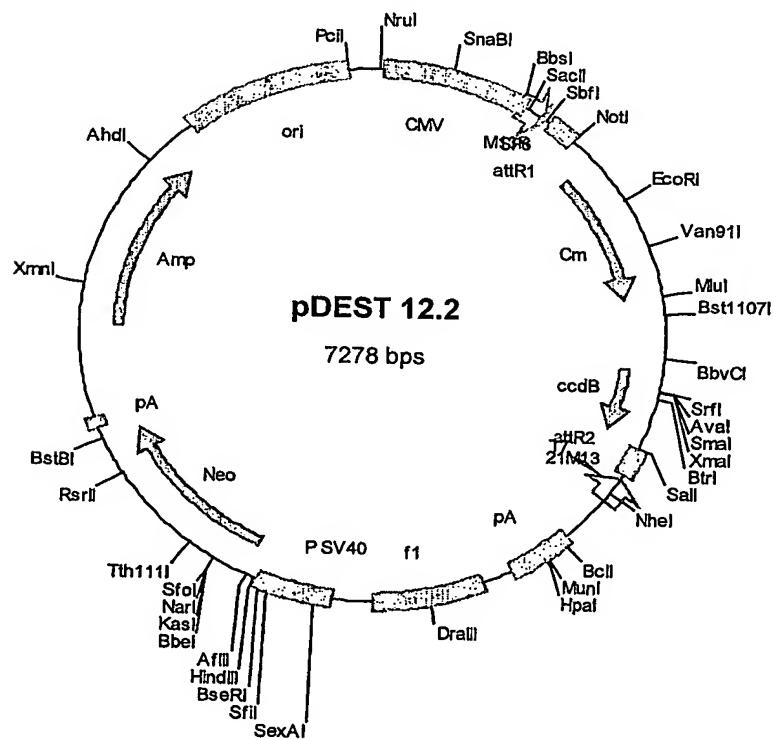


Figure 11: Map of pENTR-INSP094-6HIS

Molecule: pDONR221-INSP094-6HIS-V1, 2832 bps DNA Circular
 File Name: pDONR221-INSP094-6HIS-V1.cm5

Type	Start	End	Name	Description
REGION	295	268	C rrnB T2	transcription termination sequence
REGION	470	427	C rrnB T1	transcription termination sequence
REGION	536	553	21M13	21M13 primer
REGION	570	651	attL1	
GENE	677	952	INSP094-6HIS-V1	
REGION	967	1055	attL2	
REGION	1113	1096	C M13 R	M13R primer
GENE	1226	2035	Kan r	
GENE	2156	2829	pUC ori	

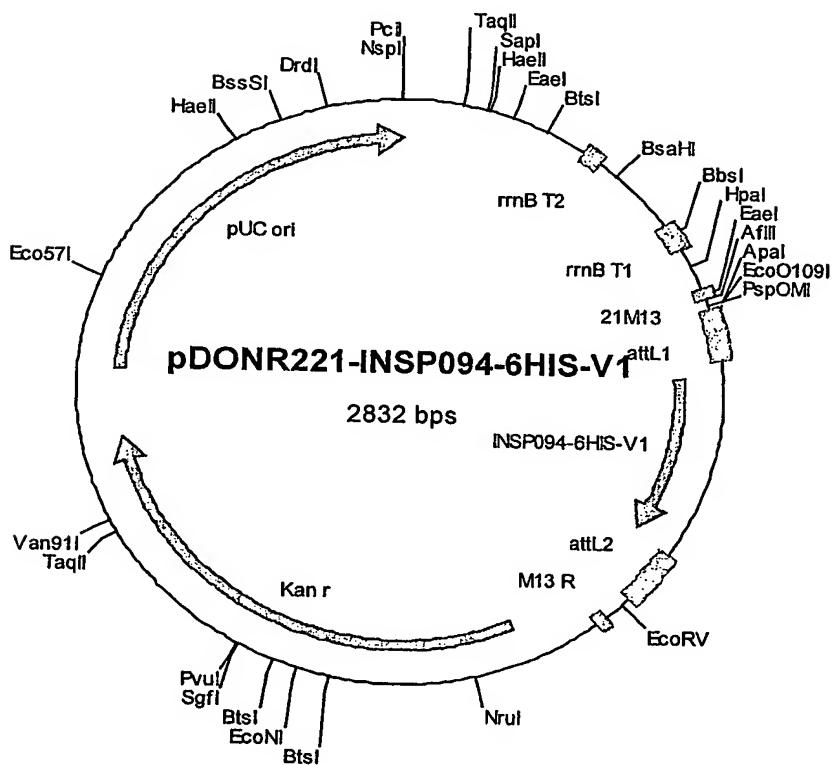


Figure 12: Map of pEAK12d-INSP094-6HIS

Molecule: pEAK12d-INSP094-6HIS-V1, 7225 bps DNA Circular
 File Name: pEAK12d-INSP094-6HIS-V1.cm5

Type	Start	End	Name	Description
REGION	2	595	pmb-ori	
GENE	596	1519	Amp	
REGION	1690	2795	EF-1alpha	
REGION	2703	2722	peak12-F	forward primer
REGION	2855	2874	attB1	
GENE	2888	3163	INSP094-6HIS-V1	
REGION	3171	3192	attB2	
REGION	3199	3627	'A	poly A/splice
REGION	3313	3294	peak12-R	reverse primer
GENE	4246	3628	PUR	PUROMYCIN
REGION	4470	4247	tK	tK promoter
REGION	4965	4471	Ori P	
GENE	7017	4965	EBNA-1	
REGION	7018	7217	sv40	

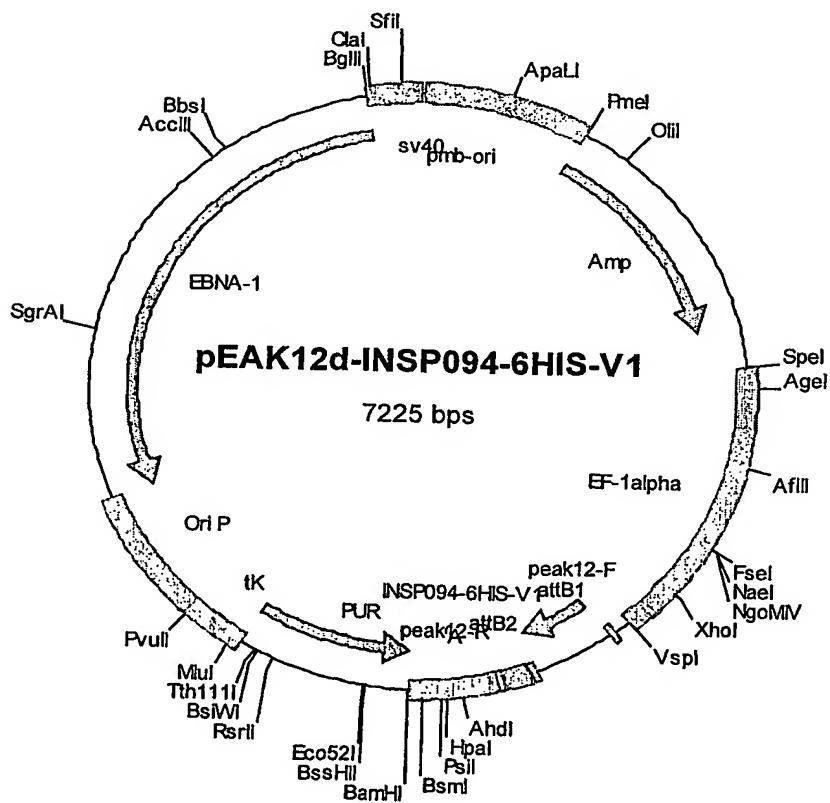


Figure 13: Map of pDEST12.2-INSP094-6HIS

Molecule: pDEST12.2-INSP094-6HIS-V1, 5911 bps DNA Circular
 File Name: pDEST12.2-INSP094-6HIS-V1.cm5

Type	Start	End	Name	Description
GENE	15	537	CMV promoter	
REGION	648	665	M13rev	M13R primer
REGION	687	704	SP6	SP6 primer
REGION	730	762	attB1	
GENE	763	1038	INSP094-6HIS-V1	
REGION	1042	1067	attB2	
REGION	1117	1097	C T7	T7 promoter
REGION	1146	1128	C 21M13	21M13 primer
GENE	1252	1614	pA	SV40 polyadenylation signal
GENE	1808	2264	f1	f1 intergenic region
GENE	2328	2746	P SV40	SV40 ori & early promoter
GENE	2791	3585	Neo	
GENE	3649	3697	pA	poly adenylation signal
GENE	4108	4968	Amp	
GENE	5117	5756	ori	pUC ori

